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DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

AD-A221 845

1b. RESTRICTIVE MARKINGS: N/A

2a. SECURITY CLASSIFICATION AUTHORITY: N/A

3. DISTRIBUTION/AVAILABILITY OF REPORT: Distribution Unlimited

2b. DECLASSIFICATION/DOWNGRADING SCHEDULE: N/A

4. PERFORMING ORGANIZATION REPORT NUMBER(S): Kansas State University

5. MONITORING ORGANIZATION REPORT NUMBER(S): N/A

DTIC ELECTE MAY 13 1990 D

6a. NAME OF PERFORMING ORGANIZATION: Kansas State University

6b. OFFICE SYMBOL (If applicable): N/A

7a. NAME OF MONITORING ORGANIZATION: Office of Naval Research

6c. ADDRESS (City, State, and ZIP Code): Division of Biology, Ackert Hall, Manhattan, KS 66506

7b. ADDRESS (City, State, and ZIP Code): 800 No. Quincy Street, Arlington, VA 22217-5000

8a. NAME OF FUNDING/SPONSORING ORGANIZATION: Office of Naval Research

8b. OFFICE SYMBOL (If applicable): ONR

9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: N00014-87-K-0217

8c. ADDRESS (City, State, and ZIP Code): 800 No. Quincy Street, Arlington, VA 22217-5000

10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.
61153N	RR04108	441f722	

11. TITLE (Include Security Classification): (U) The role of neuropeptides in persistent virus infections of the central nervous system.

12. PERSONAL AUTHOR(S): Johnson, Terry C.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
FINAL	FROM 3/1/87 TO 11/30/89	May 10, 1990	19

16. SUPPLEMENTARY NOTATION

17. COSATI CODES		
FIELD	GROUP	SUB-GROUP

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number): opioids, lymphocytes, infections, nervous system, virus, immunity, neuropeptides

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

A temperature-sensitive (ts) mutant of vesicular stomatitis virus (VSV), tsG31 KS5, engendered a slowly progressive paralytic central nervous system (CNS) disease that killed all Balb/c nude mice within 28 days. Reconstitution of nude mice with 10^7 syngeneic splenocytes 24 h before intracerebral inoculation with tsG31 KS5 VSV, however, protected 92% of the animals from death. When these reconstituted animals were injected intracerebroventricularly with 14 pmol of β-endorphin 24 h after reconstitution with splenocytes and 24 h before inoculation with tsG31 KS5 VSV, only 72% of the animals survived. Furthermore, whereas 40% of the afflicted reconstituted nude mice given intracerebroventricular injections of sterile water were able to recover from the symptoms of disease, those surviving animals which received β-endorphin were unable to

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT: ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS

21. ABSTRACT SECURITY CLASSIFICATION: (U)

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
Dr. J.A. Majde	(202) 696-4055	ONR

DD Form 1473, JUN 86 — Previous editions are obsolete. — SECURITY CLASSIFICATION OF THIS PAGE

90 05 12 089

19. ABSTRACT (cont.)

do so. A single intravenous injection of 14 pmole β-endorphin, or repeated postinfection administration of 28 pmol of β-endorphin intravenously into nude mice reconstituted with syngeneic splenocytes, which were pretreated with β-endorphin, did not alter the course of CNS disease induced by tsG31 KS5 VSV. The effect induced by intracerebroventricular injection of

β-endorphin was antagonized by naloxone, but not by the neuropeptide fragment β-endorphin-(1-27). A simultaneous intacerebroventricular injection of reconstituted nude mice with 1220 pmol of naloxone and 14 pmol of β-endorphin resulted in a 89% survival rate, and 33% of the afflicted animals were able to overcome the symptoms of the disease induced by tsG31 KS5 VSV. Intracerebroventricular injection of reconstituted nude mice with 330 pmol of β-endorphin-(1-27) and 14 pmol of β-endorphin resulted in a 72% survival rate and the surviving animals were unable to improve appreciably the clinical status of their disease. Injection of reconstituted nude mice with either 1220 pmol of naloxone or 330 pmol of β-endorphin-(1-27) alone did not alter the course of the CNS disease in any way. A single intracerebroventricular injection of 29 pmol of another psychoactive peptide, des-tyr-endorphin, 24 h after reconstitution of nude mice with splenocytes and 24 h prior to infection with virus, resulted in 74% survival; and 39% of the afflicted animals were able to recover from the clinical symptoms.

FINAL REPORT

The Role of Neuropeptides in Persistent Virus Infections of the Central Nervous System

ONR Contract No. N00014-87-K-0217

A. Introduction

β-endorphin has been shown to have many different effects on immune cells. On the cellular level, the effects of β-endorphin on immune cells are mediated via two different classes of receptors. Through opioid receptors, β-endorphin enhances the cytotoxicity of natural killer cells (Matthews et al., 1983) and mediates chemotaxis of human blood mononuclear cells (Van Epps and Saland, 1984; Ruff et al., 1985). Through non-opioid receptors (Hazum et al., 1979; Schweigerer et al., 1982), β-endorphin suppresses stimulation of lymphocytes (McCain et al., 1982; Deitch et al., 1988), and has been shown to enhance proliferation of lymphocytes (Gilman et al., 1982).

Although evidence points to a potential role for β-endorphin in modulating immune responses, there is a paucity of information concerning the effect of β-endorphin on the overall clinical progress of infectious disease in vivo. We have demonstrated that β-endorphin dramatically altered the course of CNS disease in two genetically different strains of mice (Doll and Johnson, 1989). β-endorphin, however, was unable to alter the course of CNS disease in Balb/c (nu/nu) nude mice (Doll and Johnson, 1989). Nude mice have no thymus and, thus, lack a full repertoire of mature, differentiated T-cells (Kindred, 1981). Therefore, we reasoned that the ability of β-endorphin in altering the course of CNS disease in Balb/c mice may have been due to an immune-neuropeptide interaction, and the failure of β-endorphin to do so in nude mice may have been due to a lack of appropriate immune targets for neuropeptide action. However, nude mice reconstituted with syngeneic splenocytes, which are rich in mature T-cells, and infected with ts VSV should be susceptible to the action of β-endorphin and should undergo a more severe clinical disease. In this **final report** we show that a single intracerebroventricular injection of β-endorphin into nude mice can indeed alter the course of CNS disease induced by ts VSV. We also explore with analogs of β-endorphin the specificity of the response and the possible receptors through which this effect might be mediated.

B. Research Results

1) Materials and methods

a. Subjects

Balb/c (+/+) and Balb/c nude (nu/nu) mice were acquired from Harlan Sprague Dawley (Indianapolis, IN, U.S.A.). Three to four week old male Balb/c nude were used, and all the mice had free access to sterile food and water. The mice were maintained throughout the experiments in an animal Stay-Clean unit from Lab products (Maywood, NJ, U.S.A.).

b. Virus and inoculations

TsG31 KS5 VSV was a double-cloned isolate, plaque purified from tsG31 VSV, purified on sucrose gradients, and stocks were prepared as described previously (Rabinowitz et al., 1976). Mice were lightly anesthetized with ether and infected intracerebrally with 100 plaque forming units (pfu) of virus in 30 μl of Hanks' balanced salt solution (HBSS).

c. Injection of neuropeptides

Prior to injection, β-endorphin, β-endorphin-(1-27), and des-tyr-endorphin (synthetic human; Sigma Chemical Co., St. Louis, MO, U.S.A.) and naloxone (Sigma Chemical Co., St. Louis, MO, U.S.A.) were solubilized in sterile, distilled water. The amounts per mouse of β-endorphin, 14 pmol; β-endorphin-(1-27), 330 pmol; des-tyr-endorphin, 29 pmol; and naloxone, 1220 pmol, were diluted in 10 μl of sterile, distilled water. The animals were lightly anesthetized with ether and the neuropeptides were injected intracerebroventricularly with a Hamilton syringe equipped with a 30 gauge needle. Control animals received 10 μl of sterile, distilled water.

d. Splenocytes

Syngeneic splenocytes were isolated from the spleens of 2 month old Balb/c (+/+) mice by nylon wool separation as described previously (Julius et al., 1973). Briefly, the spleens were removed from Balb/c (+/+) by sterile dissection, and were suspended by pressing them through a fine stainless steel mesh submerged in HBSS. The cell suspensions were centrifuged (250 g for 3 min) and resuspended in cold Tris-buffered isotonic ammonium chloride to lyse erythrocytes. After a 10 min incubation at 0°C, the splenocytes were washed 3 times with HBSS, resuspended in Dulbecco's modified eagle medium supplemented with 10% fetal calf serum (DMEM/FCS), then were incubated on 30 ml nylon wool columns for 1 hr at 38°C. The splenocytes were collected from the columns in DMEM/FCS, and washed once in HBSS. After washing, the cells were resuspended in a concentration of 10^8 per ml then 100 μl were injected intravenously into the tails of nude mice with a 30 gauge needle.

e. Clinical status of disease

Mice were checked daily for the duration of the experiments and they were graded by examination on a scale from 0 to 5, with grade 0 representing complete health, grade 1 representing initial signs of disease, grade 2 as animals with difficulty in walking or a clumsy gait, grade 3 as those with one hind limb paralyzed, grade 4 representing as animals with both hind limbs paralyzed, and grade 5 representing death. Animals at grade 2 or above were considered to be diseased, and only those animals at grade 2 or above that improved to grades 1 or 0 were considered to have recovered from the disease.

2) Experimental data

Balb/c nude mice infected with 100 pfu of tsG31 KS5 VSV undergo a CNS disease that resulted in wasting, loss of appetite, hindlimb paralysis and, ultimately, in the death of all the animals within 28 days. These animals, however, can be protected by reconstitution with syngeneic splenocytes. Reconstitution of nude mice with 10^7 nylon wool nonadherent syngeneic splenocytes prior to infection resulted in the protection of 92% of the animals from death (Fig. 1).

Since we were able to protect the animals from death, we next wanted to investigate the effect of β-endorphin on the course of CNS disease as measured by survival in reconstituted nude mice infected with tsG31 KS5 VSV. A single injection of 14 pmol of β-endorphin (50 ng) administered intracerebroventricularly into reconstituted nude mice altered the course of clinical disease induced by tsG31 KS5 VSV, causing 28% more animals to die than those animals which received injections of sterile water (Fig. 2). This effect was consistently repeatable, and analyses of eight independent experiments revealed there to be a significant statistical difference between the experimental and control populations -- the p values from a one-tailed, unpaired, t-test at days 15 and 28 postinfection were 0.007 and 0.01, respectively. Increasing the level of β-endorphin from 14 pmol to 28 pmol or 140 pmol, however, had the same degree of influence on the course of disease and on the amount of death induced (data not shown). Unlike the enduring hypothermia that we previously had reported with 28 pmol (100 ng) of β-endorphin (Doll and Johnson, 1989), 14 pmol of β-endorphin did not produce a lasting hypothermia in Balb/c mice (Table 1), indicating that hypothermia was not responsible for the increased virulence.

In contrast to injection of β-endorphin into the brain, a single intravenous injection of 14 pmol of β-endorphin into nine nude mice 24 h before reconstitution with 10^7 splenocytes, and 24 h prior to infection with 100 pfu tsG31 KS5 VSV, did not alter the course of CNS disease. Eighty-nine percent of the animals in this experiment survived the virus infection (data not shown). Furthermore, pretreatment of splenocytes with β-endorphin and re-

peated intravenous injections of β-endorphin also did not alter the ability of nude mice to survive the CNS disease induced by tsG31 KS5 VSV. Syngeneic splenocytes, 10^8/ml of HBSS, were pre-incubated with 28 pmol of β-endorphin for 30 min at 25°C, washed 3 times with HBSS and 10^7 cells in 100 μl of HBSS were then intravenously injected into nude mice. Two days later, the mice were infected intracerebrally with 100 pfu tsG31 KS5 VSV, and they were given repeated intravenous injections of 28 pmol of β-endorphin in 100 μl of HBSS every other day up to 7 days post-infection. All of the animals survived (data not shown).

To further elucidate the effect of β-endorphin on the CNS disease induced by tsG31 KS5 VSV on reconstituted nude mice, we daily monitored, throughout the experiment, the clinical symptoms of animals injected with either sterile water or β-endorphin. By day 12 postinfection, surprisingly, approximately the same amount of disease was engendered in both the group of animals receiving sterile water and the group receiving 14 pmol of β-endorphin. However, whereas 40% of the afflicted animals receiving intracerebroventricular injections of sterile water recovered by day 32, the surviving afflicted animals which received 14 pmol intracerebroventricular injections of β-endorphin did not appreciably recover from the disease (Fig. 3).

To investigate the possibility that the effect was being mediated through opioid receptors, we employed naloxone, the classic opioid antagonist, and β-endorphin-(1-27), a naturally occurring fragment of β-endorphin that can be used to antagonize β-endorphin. Reconstituted nude mice were injected intracerebroventricularly with either 1220 pmol of naloxone alone or simultaneously with 1220 pmol of naloxone and 14 pmol of β-endorphin. Similar to sterile water controls, 90% of the animals receiving both β-endorphin and naloxone survived challenge with 100 pfu tsG31 KS5 VSV (Table 2), and two-thirds of those afflicted animals were able to ameliorate the clinical status of their disease (Table 3). All of the animals receiving only naloxone survived the disease, and 33% of those afflicted were able to recover from the disease (Table 2).

Reconstituted nude mice also were injected intracerebroventricularly with either 330 pmol of β-endorphin-(1-27) or simultaneously with 330 pmol of β-endorphin-(1-27) and 14 pmol of β-endorphin. Unlike naloxone, however, β-endorphin-(1-27) was unable to antagonize the effects of β-endorphin as determined by survival and the clinical status of disease induced by tsG31 KS5 VSV in reconstituted nude mice. Similar to animals receiving only 14 pmol of β-endorphin, 28% of the animals receiving both β-endorphin and β-endorphin-(1-27) succumbed to the disease (Table 2), and these animals were unable to improve appreciably the clinical status of their disease (Table 3). All of the animals receiving only β-endorphin-(1-27) survived the disease (Table 2), and nearly one-fourth of those afflicted were able to ameliorate the clinical status of their disease (Table 3).

In order to determine whether the observed effect of β-endorphin on the course of CNS disease in reconstituted nude mice was specific to β-endorphin alone or perhaps to a more general phenomenon of psychoactive neuropeptides, we employed des-tyr-endorphin, a naturally occurring psychoactive peptide composed of the 2nd through 15th amino acids of β-endorphin. When 29 pmol of des-tyr-endorphin was injected intracerbroventricularly into reconstituted nude mice 24 hours prior to infection with tsG31 KS5 VSV, des-tyr-endorphin increased the lethality of the disease very similar to that observed with β-endorphin (Table 2). However, unlike β-endorphin, the surviving animals receiving des-tyr-endorphin were able to recover from the disease as determined by clinical symptoms. With respect to this criterion, they responded very much like animals receiving sterile water injections as 39% of the afflicted animals injected with 29 pmol of des-tyr-endorphin were able to recover from the disease (Table 3).

3) Discussion

Just 100 pfu of tsG31 KS5 VSV, a more natural dose of virus, was able to induce an infection similar to that which we previously had reported with 10^4 pfu of the same virus (Doll and Johnson, 1989). Nude mice infected with 100 pfu tsG31 KS5 VSV underwent a slowly progressive disease that killed all the mice within 28 days. Reconstitution of nude mice with 10^7 syngeneic splenocytes, however, protected 92% of the animals from death (Fig. 1). When these reconstitituted animals were intracerebroventricularly injected with β-endorphin 24 hours prior to infection with tsG31 KS5 VSV, an alteration in the course of disease occurred. β-endorphin caused death in 28% of the animals and it also prevented the surviving afflicted animals from ameliorating the clinical status of their disease.

It is apparent, however, that hypothermia was not responsible for the effect of β-endorphin on the course of CNS disease induced by tsG31 KS5 VSV as we had previously believed. First, the dose of 50 ng of β-endorphin used in the present study altered the course of CNS disease without producing any hypothermia (Table 1). Secondly, previous results from our lab also indicated no detectable effect of β-endorphin on the CNS disease in unreconstituted nude mice even though the 100 ng dose given them produced a lasting hypothermia (Doll and Johnson, 1989). Furthermore, it is evident that, since β-endorphin had no effect on the course of CNS disease in unreconstituted nude mice (Doll and Johnson, 1989), but did in reconstituted nude mice, β-endorphin must have elicited its effect, either directly or indirectly, on the immune system, and an indirect effect of β-endorphin on the immune system is most likely. We observed no effect on the course of CNS disease induced by tsG31 KS5 VSV with a single intravenous injection of β-endorphin, or when β-endorphin was applied directly to splenocytes before reconstitution of nude mice and then was repeatedly injected intravenously into these same animals for up to 7 days postinfection.

In order to determine whether the action of β-endorphin was mediated through opioid receptors, we employed the classic opioid antagonist naloxone. Naloxone has greatest affinity for the μ opioid receptor, but at high concentrations, i.e. a molar ratio of 85 nmol naloxone to 1 nmol β-endorphin, it will block both the μ and δ opioid receptors (Hammonds et al., 1987), which are the opioid sites to which β-endorphin binds. At this ratio, naloxone did block the effect of β-endorphin on the course of CNS disease induced by tsG31 KS5 VSV, as determined by survival and the ability of afflicted animals to recover from disease, indicating that the effect was mediated through opioid receptors. Naloxone alone did not alter the course of CNS disease induced by tsG31 KS5 VSV.

Surprisingly, we could not block the effect of β-endorphin with β-endorphin-(1-27), a naturally occurring fragment of β-endorphin that has been reported to bind the same μ and σ opioid receptors as does β-endorphin (Nicholas et al., 1985) and to be 4 times more potent in antagonizing β-endorphin's opiate effects (Nicholas and Li, 1984; Suh et al., 1987; Bals-Kubik et al., 1988). Why naloxone should block the effect of β-endorphin and β-endorphin-(1-27) would not, is unclear. One possible explanation is that studies using β-endorphin-(1-27) as an antagonist of β-endorphin have been limited to 3 hours (Suh et al., 1987); thus, β-endorphin-(1-27) may be less stable than naloxone and, unable to maintain its antagonism throughout the 24 h between injection of neuropeptide and challenge with virus in our model. All of the animals receiving β-endorphin-(1-27) alone survived challenge with tsG31 KS5 VSV and were able to ameliorate appreciably the clinical status of their disease, i.e. they behaved very much like animals receiving intracerebroventricular injections of sterile water.

It is apparent from the results obtained from animals injected only with either naloxone or β-endorphin-(1-27), that merely occupying the opioid receptor does not elicit an effect on the course of CNS disease induced by tsG31 KS5 VSV, i.e. only a psychoactive molecule will perform. Des-tyr-endorphin, a psychoactive peptide, altered the course of CNS disease induced by ts G31 KS5 VSV. Des-tyr-endorphin corresponds to the 2nd through 15th amino acids of β-endorphin, and has been shown to extinguish active avoidance behavior, to attenuate passive avoidance behavior in rats (De Wied et al., 1978), and to ameliorate symptoms of schizophrenia (De Jongh et al., 1982). A single intracerebroventricular injection of 29 pmol of des-tyr-endorphin lowered the amount of survival of reconstituted nude mice challenged with ts G31 KS5 VSV, which was similar to the effect observed with β-endorphin on survival of reconstituted nude mice from the CNS disease induced by tsG31 KS5 VSV. Unlike animals receiving β-endorphin, however, the surviving animals receiving des-tyr-endorphin were able to improve the clinical status of their disease (Table 3). Why des-tyr-endorphin elicited its effect on survival like β-endorphin, but not on the clinical status of the CNS disease, may be that des-tyr-endorphin mediated its effect via some other mechanism. It has been shown that removing the N-terminal amino

acid tyrosine from met-enkephalin, which corresponds to the first five amino acids of β-endorphin, abolishes met-enkephalin's ability to bind to opioid receptors (Frederickson, 1977). Since des-tyr-endorphin is missing this same amino terminus, it also must be unable to bind to the opioid receptor (De Wied et al., 1978).

Considerable anecdotal evidence exists which suggests a role for neuropeptides on the immunomodulation of disease. Clinicians have long noticed that opiate addicts have increased risk and severity of infections (Briggs et al., 1967; Hussey and Katz, 1950; Olsson and Romansky, 1962). Clinical evidence from mice has also suggested that stress, which is modulated by neuropeptides (Stein et al., 1985; Grossman, 1988), can increase susceptibility to infection (Riley, 1981). The implicit message in this evidence is that neuropeptides may alter host immunity to infection, but proving such a link has been difficult.

The work done on elucidating the interaction between the neuroendocrine and immune systems during the course of a disease has been approached in two different ways -- studying the effects of neuropeptides on immunocytes and studying the effects of neuropeptides on the course of infectious disease. In the first approach, many in vitro and in vivo studies have shown that neuropeptides have an effect on immunocytes. As examples, β-endorphin has been shown to enhance (Gilman et al., 1982) and suppress (McCain et al., 1982) proliferation of mitogen stimulated lymphocytes, and to bind to the terminal complex of human complement (Schweigerer et al., 1982); Met-enkephalin has been shown to stimulate human blood mononuclear cell chemotaxis (Van Epps and Saland, 1984), and to suppress and enhance immune response in rats (Jankovic and Maric, 1987). Bombesin has been found in human mononuclear cells and in alveolar macrophages (Wiedermann et al., 1986). Lymphocytes also appear to be able to produce endorphins, lending further evidence that neuropeptides may serve as messengers between the immune and nervous systems (Smith et al., 1985).

The second approach has used an infected murine model to show that injection of a neuropeptide can influence the course of a disease in vivo. This was accomplished first by Doll and Johnson (1985; 1989) who showed that neurotensin and β-endorphin altered the course of disease induced by temperature-sensitive VSV in Swiss outbred and Balb/c mice. Since then, Faith et al. (1987) were able to lessen the severity of disease induced by herpes simplex virus type 2 in A/J mice. In a similar manner Huprikar et al. (1988) used bombesin to alter the course of disease induced by a temperature-sensitive isolate of VSV.

Neither of these two approaches, however, demonstrated a firm link between the neuroendocrine and immune systems in the host response to disease. Those demonstrations of the effects of neuropeptides on immunocytes were in the absence of a disease process, while those experiments showing an effect of neuropep-

tides on the course of a disease offered no experimental evidence as to whether or not the effect was due to an immune-neuropeptide interaction. The present study, provides a model which demonstrates that some form of communication indeed does exist between the neuroendocrine and immune systems that influences the clinical course of an infectious disease. First, β-endorphin exerted an effect on the immune system of reconstituted nude mice, although it was previously shown to be unable to do so in unreconstituted nude mice (Doll and Johnson, 1989). Second, the effect appeared to be indirect, since a single intravenous injection of β-endorphin or incubation of splenocytes with β-endorphin, followed by intravenous injection of β-endorphin in the reconstituted animal, did not influence the course of disease. And third, this effect was mediated via opioid receptors, since the effect was naloxone reversible.

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Figure legends

Fig. 1. Survival of nude mice infected with tsG31-KS5 VSV. Mice were infected intracerebrally with 100 pfu of virus one day after reconstitution with 10^7 syngeneic splenocytes (0), or were infected with 100 pfu of virus only (0). The reconstituted group contained 54 animals (eight experiments), and the unreconstituted group contained 25 (four experiments).

Fig. 2. Survival of reconstituted nude mice treated with β-endorphin before infection with tsG31-KS5 VSV. Mice were injected intracerebroventricularly with 14 pmol β-endorphin 24 h after reconstitution with 10^7 syngeneic spenocytes and 24 h prior to infection with 100 pfu tsG31-KS5 VSV (0). Or reconstituted nude mice were given intracerebroventricular injections of sterile water and infected as above (0). Each group contained 18 mice (two experiments), and the vertical bars represent the standard errors. At 15 days and 28 days postinfection, $p = 0.04$ as measured by a one-tailed, unpaired t-test.

Fig. 3. Intracerebroventricular injection of β-endorphin prevents reconstituted nude mice from ameliorating the clinical status of the disease induced by tsG31-KS5 VSV. Mice received either an injection of 10 μl of sterile water (0) or 14 pmol β-endorphin (0) 24 h after reconstitution with syngeneic splenocytes and 24 h prior to challenge with 100 pfu virus. The percentage of mice paralyzed or dead was plotted every 4 days for 32 days. The β-endorphin group contained 61 animals (eight experiments), and the water injected group contained 54 animals (eight experiments). The vertical bars represent the standard errors. At 24 days and 32 days postinfection, as measured with a one-tailed, unpaired t-test, $p = 0.03$ and 0.02, respectively.

TABLE 1.

EFFECT OF β-ENDORPHIN ON THE CORE TEMPERATURE OF BALB/C MICE.

Compound[a]	Amount	Time After Injection		
		0 h	12 h	24 h
		[Temperature (°C ± S.D.)][b]		
Sterile water	10 µl	38.39 ± 0.31	38.16 ± 0.49	37.69 ± 0.36
β-endorphin	14 pmol	37.48 ± 0.42	38.03 ± 0.35	37.60 ± 0.27

a Balb/c mice were intracerebroventricularly injected with the indicated amount of β-endorphin in 10 µl of sterile water or 10 µl of sterile water alone.

b The core temperatures of the mice were taken every 4 h up to 24 h.

Each group contained 10 mice.

TABLE 2.

THE EFFECT OF ANALOGS AND ANTAGONISTS OF β-ENDORPHIN ON SURVIVAL FROM CNS DISEASE

Compound[a]	Amount	Exper. (number)	Animals (number)	Days Postinfection: 5	10	15	20
				Survival (%)			
Sterile water	10 μl	8	54	100	98	96	92
Naloxone	1220 pmol	1	5	100	100	100	100
β-endorphin + naloxone	14 pmol and 1220 pmol	2	19	100	89	89	89
β-endorphin-(1-27)	330 pmol	2	23	100	100	100	100
β-endorphin + β-endorphin-(1-27)	14 pmol and 330 pmol	2	18	100	77*	72*	72*
Des-tyr-endorphin	29 pmol	3	27	97	85*	74*	74*

[a] Nude mice were injected intracerebroventricularly with the indicated amounts of analog or antagonist in 10 μl of sterile water 24 h after reconstitution with 10^7 syngeneic splenocytes and 24 h before challenge with 100 pfu tsG31 KS5 VSV.

* Indicates values significantly different from water injected control group, $p = \leq 0.03$, measured by a one-tailed, unpaired t-test.

TABLE 3.

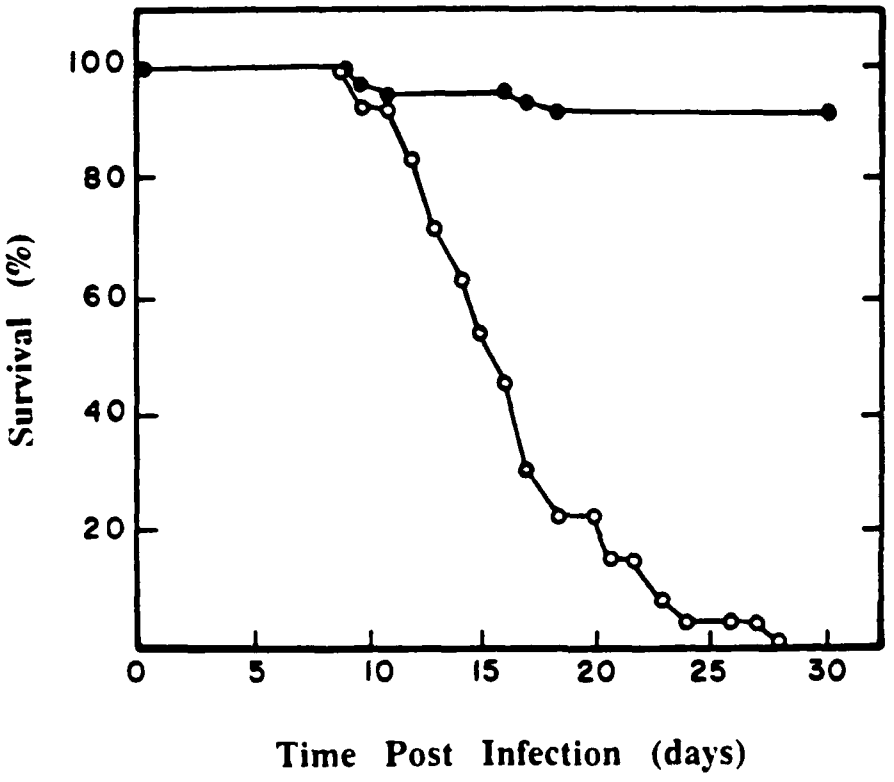
THE EFFECT OF ANALOGS AND ANTAGONISTS OF β-ENDORPHIN ON THE CLINICAL STATUS OF DISEASE.

Compound[a]	Amount	Exper. (number)	Animals (number)	Days Postinfection: Diseased (%) 8	12	16	32	Recovery[b] (%)
Sterile water	10 μl	8	54	46	66	60	40	39
Naloxone	1220 pmol	1	5	0	60	60	40	33
β-endorphin + naloxone	14 pmol and 1220 pmol	2	19	37	79	63	53	33
β-endorphin-(1-27)	330 pmol	2	23	22	65	78	60	23
β-endorphin + β-endorphin-(1-27)	14 pmol and 330 pmol	2	18	67	94	94*	89*	5
Des-tyr-endorphin	29 pmol	3	27	63	82	85*	52	39

[a] Nude mice were intracerebroventricularly injected with the indicated amounts of analog or antagonist 24 h after reconstitution with 10^7 syngeneic splenocytes and 24 h prior to inoculation with 100 pfu tsG31 KS5 VSV.

[b] Mice were monitored daily for clinical symptoms and the percent of animals paralyzed or dead was calculated. Percent recovery was determined by dividing the difference between the peak amount of disease and the amount of disease at day 32 by the peak amount of disease. Only those afflicted animals that fully overcame the paralysis were considered to have recovered from the disease.

* Indicates values significantly different from water injected control group, $p = \leq 0.03$, measured by a one-tailed, unpaired t-test.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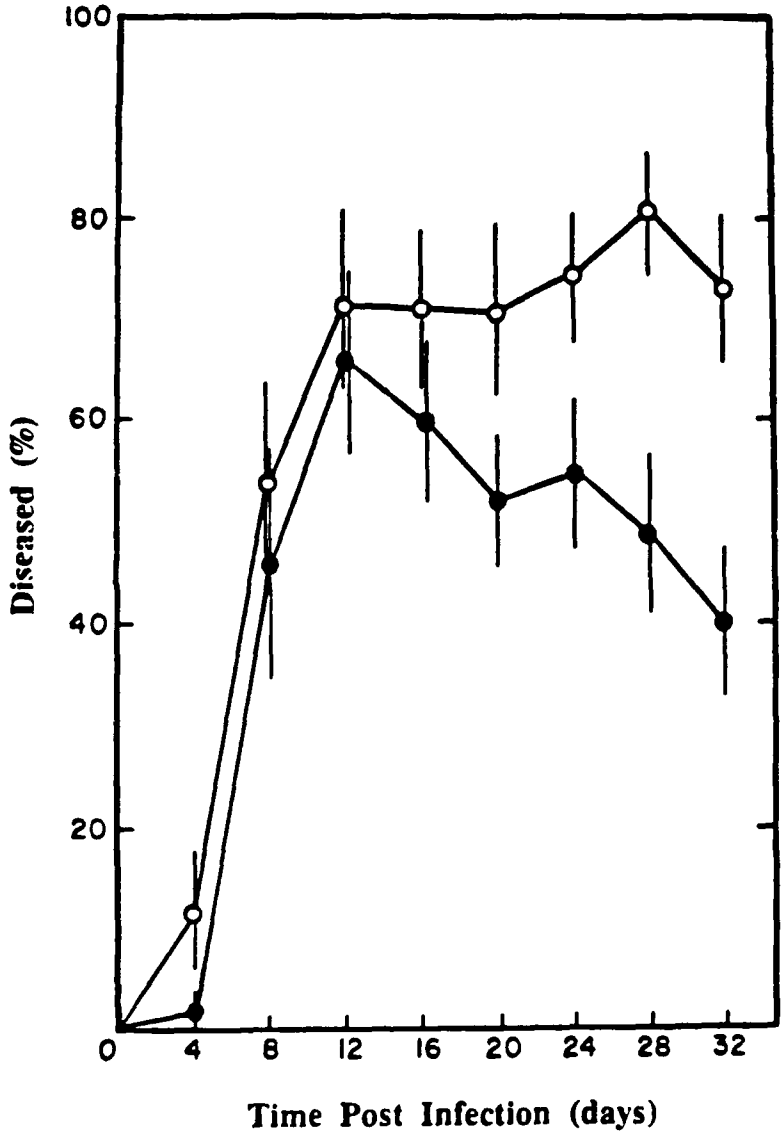
Time Post Infection (days)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Diseased (%)
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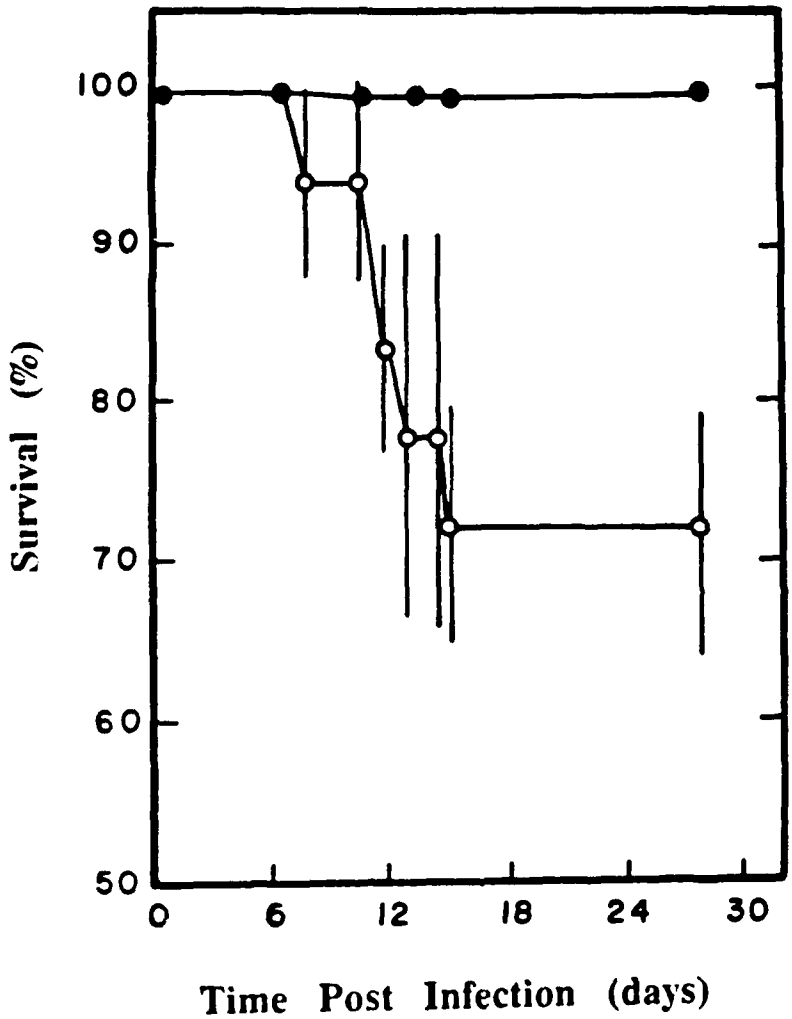
100
90
80
70
60
50
Survival (%)
0
6
12
18
24
30
Time Post Infection (days)

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